

A STAGE IN THE DEVELOPMENT OF THE SEROUS CAVITIES

W. H. McGAW

Anatomical Laboratory, Western Reserve University

ELEVEN FIGURES

The present article is the outgrowth of some elective work carried on in the anatomical laboratory some time ago with Doctor Ingalls. The intention at that time was to make a plastic reconstruction of a small human embryo in such a way as to bring out the important features in the development and gradual transformation of the body cavity. The finished model proved so clear and instructive that it has seemed worth while to put the results on record. We have chosen the method of stereoscopic photography, first, because it has become a part of the daily routine of the anatomical laboratory and, in the second place, because it is the only method of giving an adequate conception of the complicated conditions with which we are dealing.

The embryo used for reconstruction was no. 13 of the Collection of the Department of Anatomy. Its dimensions were G. L. 9.6 mm. and C. R. 8.5 mm.; these were reduced in paraffin to 8.6 mm. and 7.6 mm., respectively. The menstrual age of the specimen was about forty-four days; the real age is estimated at from thirty-seven to thirty-eight days. The reconstruction was carried out in the usual method, with wax plates, at a magnification of 50 diameters. As regards the use of color on the model we need only say that all cut surfaces are white and that all serous surfaces are gray. Details of the cut surface appear against a white background. For present purposes the important distinction is between the

white, cut surfaces and edges, and the gray, natural serous surfaces. The lines of reflection of the serous membranes have been accentuated for the sake of clearness. It should also be noted that at the upper end of the model there is a slight skewing, so that certain structures on the right are a little lower than they should be. Naturally this is least in evidence close to the median line.

Two peculiarities of this embryo may be noted here; no yolk-sac or stalk was found, and the two umbilical arteries unite within the cord.

It has not seemed necessary to make any specific references to the literature, and in the descriptions of the various views we shall concern ourselves primarily with what can be seen on the view in question, rather than attempt a detailed and connected account of the various structures and organs. Throughout these descriptions, right and left in the text refer to the right and left sides of the embryo.

A few words regarding the accompanying stereophotographs may not be out of place here. They are so arranged that the bottom of the photograph is close to the outside edge of the page. In viewing them, the card holder should be removed from the hand stereoscope and the page held in its place, level, properly centered, and at the right distance from the eyes. A smaller stereoscope, consisting simply of a mounting for a pair of prismatic lenses, would probably be somewhat easier to manage. If necessary, the page may be folded along its outer border to bring the photograph into proper position.

FIGURES

FIGURE 1

This shows the entire model in left ventrolateral view, head not included. The plane of the top section passes just above the pericardial cavity, through the larynx, and between the fifth and sixth cervical ganglia behind.

The anterior limb buds are more advanced than the posterior and are flattened distally, they lie closer to the body and are directed caudally. Segmentation, in evidence from the thoracic region backward, is most conspicuous for the lower lumbar and upper sacral somites. The tail is well developed and straight, only the tip deviating slightly to the left. The genital eminence occupies the entire interval between tail and cord, it is somewhat compressed from above downward, the caudal slope showing a well-defined median groove. Near the tip of the genital eminence a small, sagittally placed median plate rises from the floor of the groove, epithelial in character and due to proliferation in the anterior part of the thick cloacal membrane. The cord is straight and directed slightly to the right. Its cut surface shows the large, excentrically placed extra-embryonic coelom. Caudal to the coelom is the allantoic duct between the two umbilical veins, below the duct lies the umbilical artery. In this specimen, at this point, there is a single umbilical artery, the paired arteries joining in the substance of the cord.

The model of the embryonic body is made up of three pieces as shown in figure 1, an upper, middle, and lower piece. The line of separation between the upper and middle pieces is somewhat irregular. It begins ventrally in the plane of the sections just above the attachment of the cord about opposite the lower part of the seventh thoracic segment. Passing directly dorsal, under the lowest part of the pericardial cavity, it runs obliquely upward just dorsal to the lateral attachments of the septum transversum, to a point a little beyond the cephalic limit of the peritoneal cavity. Here the line of separation again becomes the plane of the sections, cutting the eighth cervical segment behind. The line between the middle and lower pieces of the model is, throughout, in the plane of section. Ventrally its relations are seen in the figure, dorsally it cuts through the lower part of the tenth thoracic segment. The window in the upper piece affords a view of the interior of the pericardial cavity. The liver is separate, fitting onto both the upper and middle pieces.

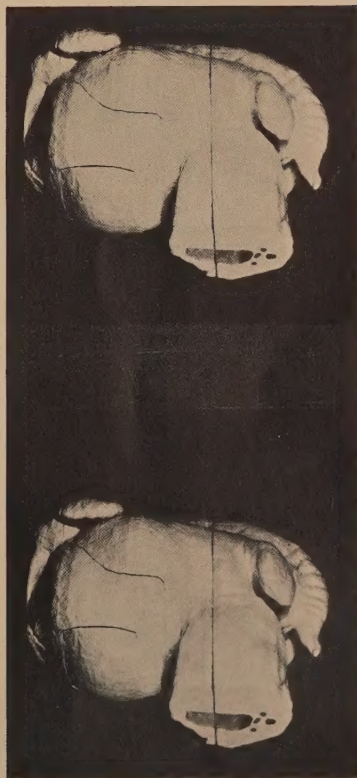


FIGURE 2

View of the roof and upper part of the posterior wall of the pericardial cavity as seen through the window in the upper piece of the model. The dorsal mesocardium consists of two portions, an anterior part or arterial mesocardium and a posterior or venous mesocardium. The former, much smaller, is high up in the roof and encloses the aorta and pulmonary artery; the aorta is on the right. The irregular cut surface below is the venous mesocardium with the cut ends of four veins. Above and on the right is the right superior cava (right duct of Cuvier), below, nearer the midline and much larger, is the inferior cava, the terminal, hepatic portion; on the left is the small left duct of Cuvier, while the single pulmonary vein is in the median line. The space between the arterial and venous mesocardia is the transverse sinus of the pericardium. The venous mesocardium occupies a little more than the upper third of the posterior pericardial wall, its high, cephalic position being due to the marked obliquity of the septum transversum (fig. 1). In the middorsal line between the mesocardia is a broad ridge which becomes more sharply demarcated below; deeply embedded within this ridge is the trachea. Immediately above the venous mesocardium and on either side of the median ridge are two small, roughly elliptical openings—the pleuropericardial passages. Between these openings appears that part of the ventral mesentery which encloses the lower end of the trachea and whose free, ventral margin looks into the pericardial cavity. Caudal to this point the ventral mesentery is attached to the dorsal border of the septum transversum; it meets the septum at a right angle and through the area of union the pulmonary vein gains the venous mesocardium and thence the left atrium. Below the septum transversum the ventral mesentery (ventral mesogastrium) is in relation with the liver (figs. 3 to 6).

The pleuropericardial openings are well up on the posterior pericardial wall, close to the roof; dorsally they open into the most anterior part of the pleural cavity close to the ventral mesentery. The left opening has a vertical diameter of 10 mm. and a transverse diameter of 8 mm. The right is slightly smaller and gives the impression of being somewhat constricted. Both openings are bounded laterally by the free, medial margins of the pleuropericardial folds; these differ somewhat on the two sides. The right fold is thicker, due to the larger size of the right cuvierian duct. The mesocardium is also drawn up on its ventral, pericardial surface. On account of the contained vein, the contribution of its dorsal surface in limiting the pleural cavity in front is rather less than on the left side. The left fold is more typical, thinner, its free edge is enlarged to enclose the small left cuvierian duct, both its ventral, pericardial and dorsal, pleural surfaces are more extensive than on the opposite side. Both pleuropericardial openings are limited internally by the lateral borders of the ventral mesentery and below by the dorsal border of the septum transversum. The dorsal surfaces of the folds are shown in figures 7 and 8.

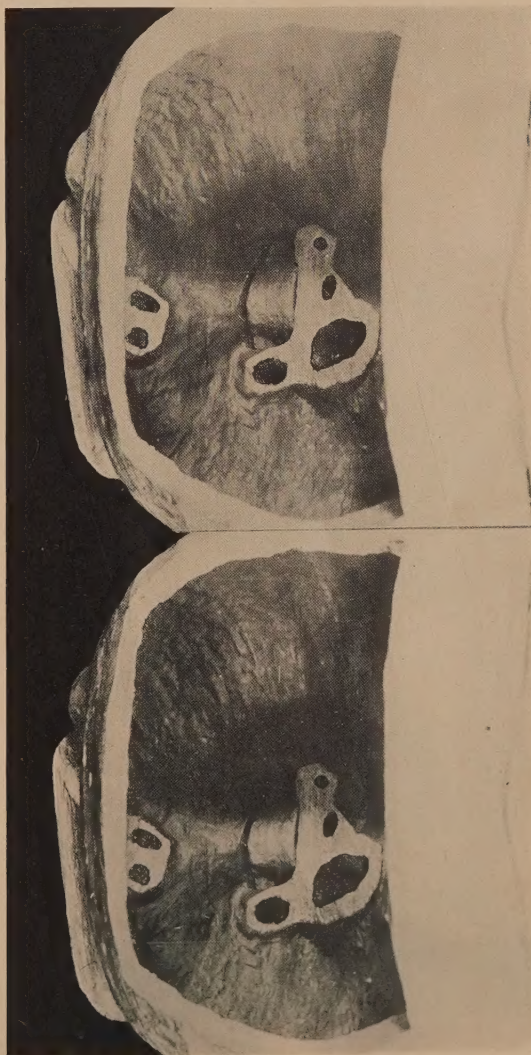


FIGURE 3

Middle segment of model, from above and in front. The liver has been removed. The upper, flat surface lies in the plane of section and passes through the eighth cervical ganglion behind. There also appear here the ventral rami of the seventh cervical nerves, notochord, the dorsal aortae just above their point of union, and sections of midoesophagus, lower trachea, and vagi. The large veins, opened by both the horizontal and vertical cuts, are the confluent anterior and posterior cardinals, the beginnings of the cuvierian ducts. Outside these veins, on the top section, are the phrenic nerves, they are cut again, more obliquely, in the vertical section near the base of the pleuroperitoneal folds.

The pleural cavities are large as compared with the lung buds and are in open communication with the peritoneal cavity below. Their lateral boundaries are formed above, by the body wall with the contained ducts of Cuvier, and below this by the inner surfaces of the pleuroperitoneal membranes which at this stage reach half-way down the lung buds. The pleuroperitoneal folds have been cut by the vertical section at the point where their ventral ends join the posterior edge of the septum transversum and the upper surfaces of the liver (figs. 6, 7, and 8). Lateral to the folds there is a small, blind pocket of peritoneum on either side. In the midline is the cut edge of the ventral mesentery throughout its entire extent. At its upper end, in the region of the pulmonary vein and to a point just below the tip of the recessus pneumato-entericus, it was connected with the dorsal border of the septum transversum (figs. 6, 7, 8). Below this level the ventral mesentery is split into two portions by the mesenteric recess or omental bursa, both of these parts are attached to the dorsal surface of the liver (figs. 8 and 9). On the left is the mesentery proper, ventral mesogastrium or lesser omentum, enclosing in its free margin below the portal vein, hepatic artery, and common bile duct. On the right of the omental bursa is the caval mesentery or mesohepar, plica venae cavae.

On the ventral body wall, above the attachment of the cord, is seen the large area over which the liver was fused with the body wall. Through this connection the two umbilical veins enter the liver from the body wall. Caudal to the liver there is a rapid constriction in the extent of body cavity.

Further details of this part of the model are shown in figures 4 and 5.

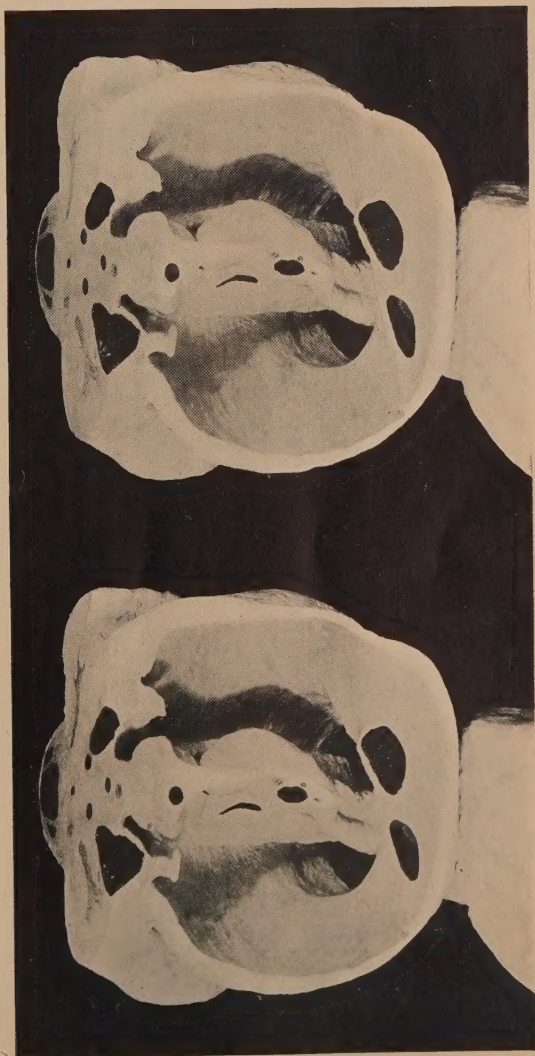


FIGURE 4

Detail of figure 3 (q.v.) from left side.

The left lung is bilobed, smaller and shorter than the right one. The free, inferior edge of the left pleuroperitoneal membrane is just above the notch between the upper and lower lobes. The lower lobe is very close to the upper end of the stomach. The left pleuroperitoneal fold has its dorsal attachment on the left side of the dorsal mesentery between the lung in front and the cephalic end of the mesonephric ridge overlying the posterior cardinal vein, behind. It describes an arc of about 90° around the lung, coming off the dorsal mesentery almost in a frontal plane, while its anterior, or ventral, attachments meet the liver and septum transversum (fig. 6) farther lateral in a sagittal plane. The free margin is concave, but with a greater radius and the dorsal end extends a little lower down than the ventral end. The phrenic nerve which appears near the base of the pleuroperitoneal membrane does not enter this fold, but makes its way into the pleuropericardial membrane (fig. 7).

The stomach has rotated a little more than 45° and is displaced to the left; its epithelial wall shows a definite spindle-shaped enlargement. A somewhat indefinite, vertical ridge on the posterior mesogastrium close to the stomach represents the spleen. A part of this elevation is just visible behind the convexity of the greater curvature. A second more sharply marked ridge (not shown), close to and parallel with root of the mesogastrium, overlies the pancreas.

Partly obscured by the stomach, deeply placed on the dorsal wall near the median line, is the mesonephros. At its extreme anterior end dorsal to the origin of the pleuroperitoneal fold, the ridge is due to the underlying posterior cardinal vein rather than to the mesonephric tissue. The latter is deeply buried here, mesial and ventral to the vein, between it and the aorta. The fine linear elevation formed by the mesonephric duct lies at first over the center of the mesonephros, but it soon gains the lateral border, which position it maintains until it reaches the terminal part of its course.



FIGURE 5

Details of figure 3 (q.v.) from the right side.

The right lung is three-lobed, larger and longer than the left. The right pleuroperitoneal membrane presents essentially the same features as the one on the left. It is somewhat lower in position, its pleural face is rather less concave and its inferior free margin is nearly straight. Its attachment to the liver appears much broader than on the left, due to the inclusion of some hepatic tissue, being cut off a little farther ventral than on the left side.

The relations of the ventral mesentery and the omental bursa are well shown. On the right, close to the mesonephros, is the caval mesentery, with a longitudinal section of the primitive vena cava inferior, i.e., the small hepatic vein which grows backward from the liver through the caval mesentery to connect with the right subcardinal vein in the posterior body wall. The recessus pneumato-entericus dexter (bursa infracardiacus) appears as a narrow slit between the oesophagus and the inferior lobe of the right lung. Below it opens out into the omental bursa, into what will later be the recessus superior omentalis. Farther back, behind the caudal half of the stomach is a second recess, the recessus mesenterico-entericus (pancreatico-entericus), which reaches to the left as far as the attachment of the dorsal mesogastrium to the greater curvature of the stomach. It forms also a deep, narrow pocket dorsal to the pyloric end of the stomach and the upper part of the duodenum, the recessus inferior omentalis. The dorsal wall of the recessus mesenterico-entericus is formed by the posterior mesogastrium in which lie the pancreas and spleen. The common opening of these various recesses, through the omental bursa into the general peritoneal cavity, is the foramen of Winslow (hiatus communis), which is seen between the inferior end of the caval mesentery and the enlarged end of the ventral mesentery (mesogastrium). In the model a metal bridge extends across the opening into the mesenterico-enteric recess.

The terminal part of the ventral mesogastrium, the ligamentum hepatoduodenale, bounding the foramen epiploicum below and on the left, contains the large portal vein with the hepatic artery on the left and the common bile duct below.

The opposite, cephalic end of the ventral mesentery, presents a free, rounded, serous surface; it contains the lower part of the trachea and looks into the pericardial cavity immediately above the septum transversum and venous mesocardium (fig. 2).

Right phrenic nerve and mesonephros, as on the left side.

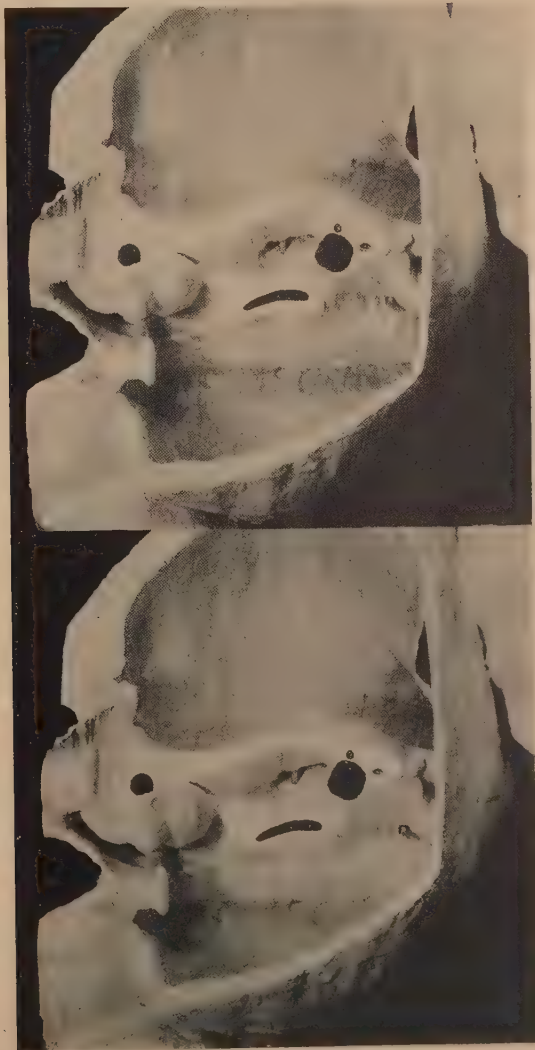


FIGURE 6

Same view as figure 3, but more from above and with liver in place.

The extensive white surface on the liver is the area of its attachment to the septum transversum (fig. 7). The large vein is the terminal hepatic part of the inferior vena cava. In the midline, immediately above the liver, is the cut edge of the ventral mesentery where it joins the septum transversum, above this the ventral mesentery is free and helps form the posterior wall of the pericardial cavity (fig. 2). The tip of the recessus pneumato-entericus appears near the right border of the ventral mesentery above the liver and below the pulmonary vein.

The ventral ends of the pleuroperitoneal folds have two attachments which are naturally continuous. The frontal sections of the folds, seen here and in the preceding figures, represent approximately the connection, in a frontal plane, with the dorsal border of the septum transversum (fig. 7). From the septum the folds continue onto the superior surfaces of the lobes of the liver with which they unite in what may be described as a horizontal plane. The cuts which separate the folds from their attachment to the liver are in a horizontal plane, so that, as seen in place, the liver is under the ventral ends of the folds. Each fold possesses a triangular cut surface, not shown, which is at right angles to its cut surface which can be seen and which represents its area of fusion with the liver. Both liver lobes project dorsally some distance beyond where they are joined by the pleuroperitoneal folds. In this way they help limit the pleural cavities below and on the outside as well as ventrally.

Both pleuroperitoneal membranes are well shown in figure 6, especially the left one, the left lobe of the liver is also seen close to the lung bud. The pleural cavities as seen here are entirely open ventrally, the closing structures are shown in figures 7 and 8.

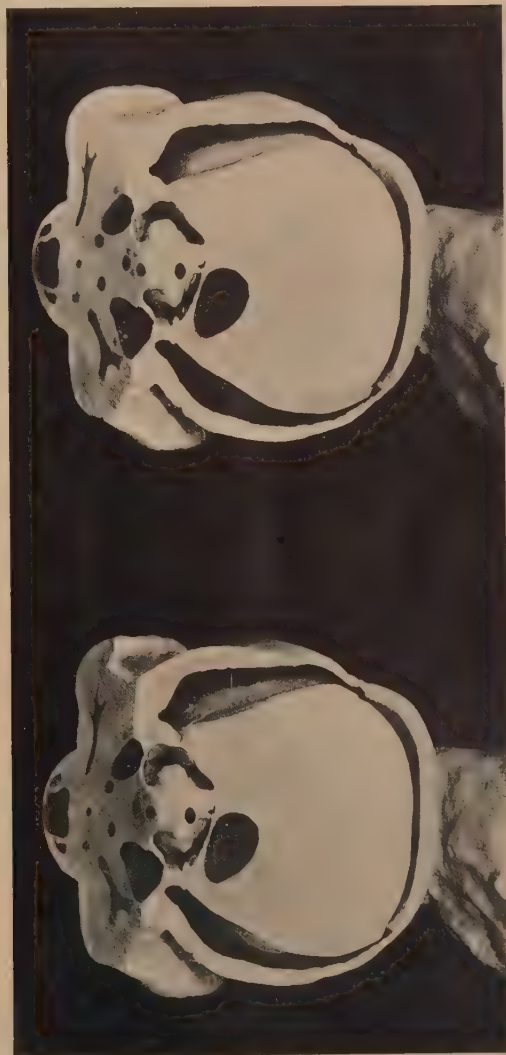


FIGURE 7

View of the upper sections of the model from behind and below, same piece as shown in figure 2. Note that the cut surfaces represented here fit onto the cut surfaces of figure 6 and also figures 3 to 5.

In the angle where the horizontal and frontal cut surfaces meet are the ducts of Cuvier, where they are formed by the union of the anterior and posterior cardinals. Outside and below are the phrenic nerves, leaving the body wall to enter the pleuropericardial membranes. In the midline, pierced by the single pulmonary vein, is the area of fusion between the dorsal border of the septum transversum and the ventral mesentery. Below and on the right, in this area, is the tip of the recessus pneumato-entericus. On either side of this median cut surface, and extending farther cephalad, are seen the ventral walls of the pleural cavities, the pleuroperitoneal passages. The caudal half, or more, of each lies in a frontal plane and is formed by the free, dorsal margin of the septum transversum. The cephalic portion of the ventral pleural wall has a more oblique position and is formed by the pleuropericardial folds, which laterally are continuous with the body wall in the region of the phrenic nerve; caudally they are fused with the septum transversum, while their internal margins are free and form the lateral boundaries of the pleuropericardial openings. The large, right duct of Cuvier can be seen entering the right fold. The large opening in the center, between the upper ends of the pleural cavities, receives the free edge of the mesentery in front of the lower end of the trachea (figs. 2 and 3). All that remain normally, then, with the mesentery in position, are the paired pleuropericardial openings, high up under the roof of the pleural cavities. The ventral surfaces of the pleuropericardial folds form a part of the dorsal wall of the pericardial cavity (fig. 2).

The entire caudal, or caudodorsal, surface of the septum transversum, except for two narrow serous strips on either side, is represented as a cut surface, since it is fused with the liver over this entire area (fig. 6). Above and on the right in this area is the inferior cava with its opening into the right atrium (fig. 2). For the purpose of description and topography, one may say that the lower, oblique sections of the phrenic nerves are located approximately at the point where the pleuropericardial and pleuroperitoneal membranes and the dorsolateral angles of the septum transversum join the lateral body wall. Just caudal to the nerves, between the upper ends of the narrow serous strips on the septum and the extreme lateral limits of the pleural cavity, there may be seen two small areas. These represent the part of the dorsal border of the septum with which the ventral ends of the pleuroperitoneal folds unite (figs. 3 to 6).

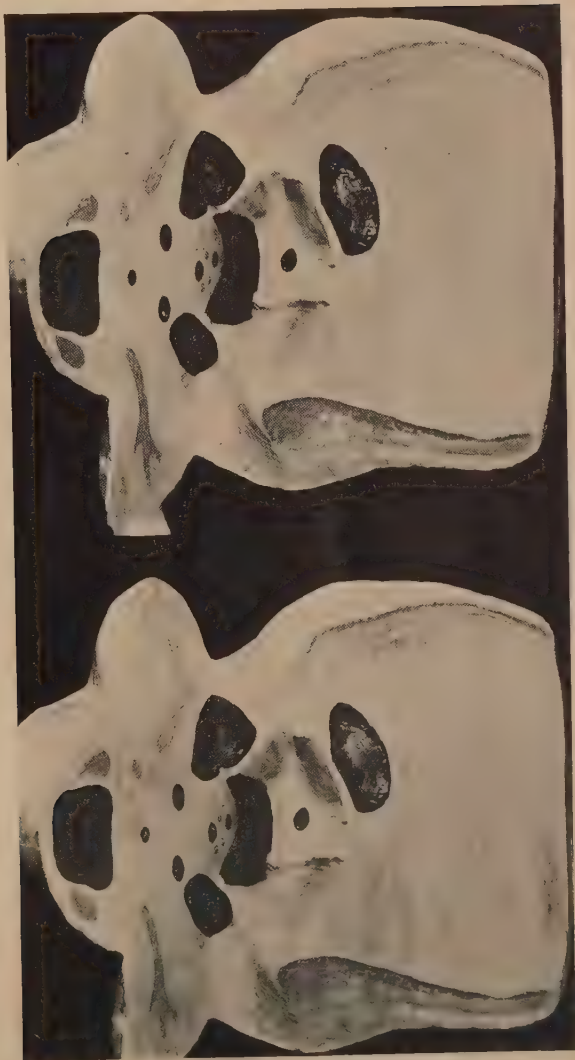


FIGURE 8

Same view as figure 7, but with the liver in place (see text to fig. 7).

The ventral mesentery is continued from the dorsal margin of the septum transversum onto the dorsal surface of the liver (fig. 3). Note the continuation of the ventral pleural wall on both sides onto the dorsal and dorsomesial surfaces of the right and left lobes of the liver. This is especially well seen on the right side where the lobe of the liver is much larger and the pulmonary impression much more distinct. These serous surfaces and also the ventral mesentery, with its two parts between them, were, in the embryo, in uninterrupted continuity. The cutting away of the liver from the septum occasions the transverse interrupting line in the model.

The pleuroperitoneal membranes, from their origins dorsal to the lungs, encircle the lungs dorsally and laterally (fig. 3); their ventral ends reach the septum transversum below the phrenic nerve and are continued beyond this onto the adjacent lobes of the liver.

For further details of liver consult text to figures 9 and 10.



FIGURE 9

Dorsal surface of liver (figs. 8, 5, and 10).

Note the deep concavity between the right and left lobes, the former is broader and much bulkier. In the midline is the attachment of the ventral mesogastrium, which terminates below, just beyond the section of the common bile duct. On the inner surface of the right lobe is the caval mesentery and the inferior cava, the beginning of the extrahepatic portion. Running forward from caval mesentery is the thin serous fold which forms the right boundary of the recessus pneumatoentericus. Immediately above the caval mesentery is the pulmonary impression, that portion of the right lobe which helps limit the right pleural cavity, while laterally is an elongated, indistinct mesonephric impression. On the left lobe the deep gastric impression is confluent above with the poorly marked pulmonary impression. On the caudal surface of the liver the two umbilical veins enter.

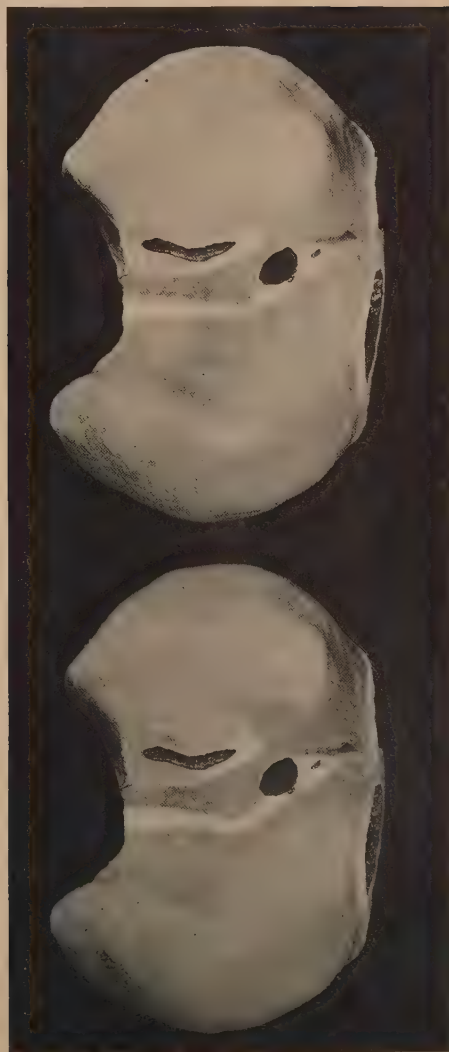


FIGURE 10

Liver from below and behind (figs. 9 and 3).

The gastric and mesonephric impression are seen better in this view. The caudate lobe already bulges into the omental bursa. Over the white area below, the liver is fused with the anterior abdominal wall just above the attachment of the umbilical cord and below the ventral limit of the septum transversum. Through this area the two umbilical veins enter the liver from the body wall.



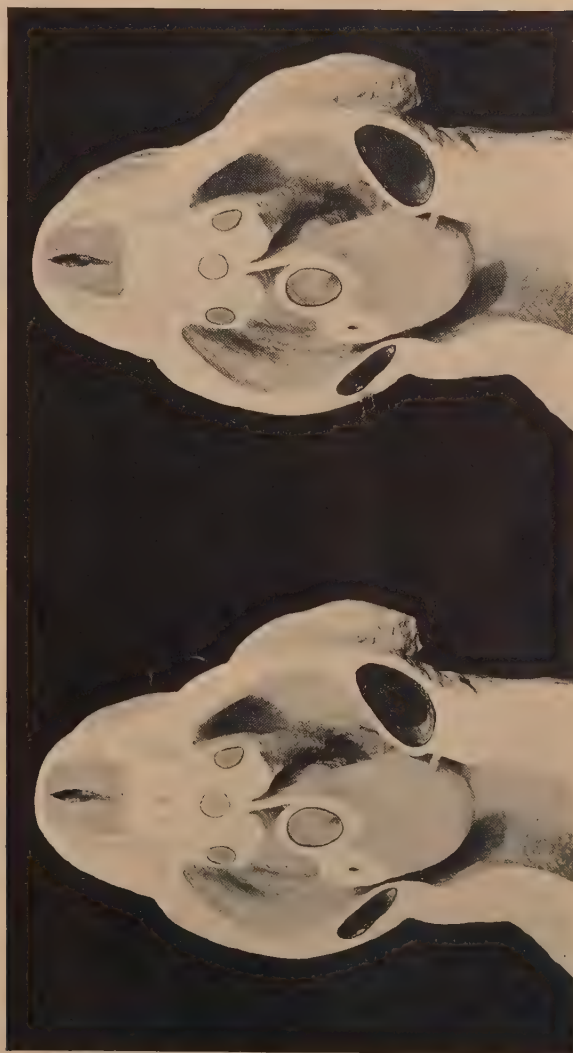
FIGURE 11

Lower segment of model, looking downward and toward the right. The plane of section passes a little above the center of the cord and through the tenth thoracic segment.

On the cut surface appear the aorta, posterior cardinal veins, mesonephric tubules, and duct. In the lateral body wall, close to the cord, are the two umbilical veins. At this level they are running directly upward to reach the liver (fig. 3).

The dorsal mesentery is thin at its attachment and encloses near its free margin the small, epithelial, intestinal tube. The descending limb of the intestinal loop, the remainder of which is shown in figure 3, is on the right; on the left is the enlarged caecal region of the distal ascending limb. The summit of the loop is within the cord, the cavity of which is in wide communication with the intra-embryonic coelom. The large vessel in the mesentery is the superior mesenteric artery. No yolk stalk was present.

The wolffian bodies taper off into the lowest part of the coelom, the ducts leave their lateral positions and come to lie ventral. As may be seen on the cut surface, only a small part of this longitudinal elevation is due to the presence of mesonephric tissue. The lowest part of the peritoneal cavity is in the form of a narrow, crescentic pouch between the intestine behind and the bladder in front. The left mesonephric duct can be seen crossing the left end of the crescent to reach the horn of the bladder.



THE VASCULAR SYSTEM IN RELATION TO NEUROMUSCULAR FUNCTIONS IN THE EARLY DEVELOPMENT OF AMBLYSTOMA

G. E. COGHILL AND JULIA MOORE

Department of Anatomy, University of Kansas¹

THIRTEEN FIGURES

The junior author has reported briefly upon her studies of the development of the blood vascular system of *Amblystoma*² (Moore, '15). These studies were undertaken not with a view to making a contribution to the knowledge of the vascular system as such, but for the purpose of making known just how the vascular system is correlated with the neuromuscular system in the development of the function of the animal as a whole according to the physiological stages described by the senior author (Coghill, '08, '09, '13 a, '13 b, '14, '16, '24, '24 a; Herrick and Coghill, '15).³

¹ For financial aid in the prosecution of this study, acknowledgment is due the Research Committee of the Graduate School of the University of Kansas.

² The anatomical descriptions relate to *Amblystoma punctatum* (collected at Granville, Ohio). The physiological observations were made upon *Amblystoma microstomum* (collected at Lawrence, Kansas). The junior author's original paper, of which the descriptive part of this contribution is a résumé, dealt comparatively with the work of other investigators upon the blood and vascular system of Amphibia (Brachet, Clark, Dekhuysen, Evans, Field, O. Hertwig, Hochstetter, Hooker, Johnson, Kingsley, Knower, Marshall and Bliss, Oellacher, Paton, Rabl, Salensky, Schwink, Shore, Torok); but since this paper is not essentially morphological, there seems to be no occasion to review this literature in detail here.

³ These stages are as follows: non-motile, characterized by muscular reaction to electrical or mechanical, but not to tactile stimuli; early flexure, marked by the earliest muscular response to tactile stimuli; coil, characterized by earliest tightly coiled movements; 'S' reaction, characterized by the earliest simultaneous contractions in opposite directions in different parts of the body; early swimming, marked by the development of the latter reaction to such strength as to produce locomotion through at least two or three body lengths.

A. GENERAL ANATOMY

1. *The non-motile stage* (fig. 1)

The vascular system of the non-motile stage is very difficult to follow because of the relatively undifferentiated condition of the blood vessels. The heart is a simple straight tube of mesoderm. It receives posteriorly the subintestinal vein (*sub.int.*) and the ductus Cuvier (*d.Cuv.*), both of which are very short, and perceptible as definite vessels only in their most basal portions. Anteriorly it extends for a short distance as a rather slender vessel, which may be regarded as the beginning of the ventral aorta (*v.a.*). A small section of the anterior cardinal vein (*int.jug.*) opposite the auditory vesicle and passing rostrally to the vicinity of the isthmus is the only blood vessel that is clearly differentiated out of the loose mesenchyme which surrounds the nervous system. The only other vessels at this stage are the endothelial spaces which later go to make up the dorsal aortae and aorta of the trunk and those spaces on the ventrolateral surface of the yolk, which at this time contain blood corpuscles.

2. *Early flexure stage* (fig. 2)

In the early flexure stage the heart has become slightly coiled. The ventral aorta (*v.a.*) extends forward and divides into two branches, but there is no suggestion of aortic arches.

ABBREVIATIONS

<i>aa</i> , 1, 2, 3, 4, aortic arches	<i>my.</i> , myotome
<i>a.i.c.</i> , internal carotid artery	<i>n.</i> , notochord
<i>cor.</i> , blood corpuscles	<i>nr.</i> , subnotochordal rod
<i>c.v.</i> , caudal vein	<i>o.p.</i> , olfactory placode
<i>d.a.</i> , dorsal aorta	<i>o.v.</i> , ophthalmic vein
<i>d.Cuv.</i> , ductus Cuvier	<i>p.c.</i> , postcardinal vein
<i>end.</i> , endothelium of heart and arteries	<i>prc.</i> , pericardial cavity
<i>ent.</i> , entoderm	<i>prn.</i> , pronephros
<i>gl.</i> , glomerulus	<i>sub.int.</i> , subintestinal vein
<i>h.v.</i> , hypogastric vein	<i>s.v.</i> , sinus venosus
<i>i.j.</i> , precardinal vein	<i>v.a.</i> , ventral aorta
<i>int.jug.</i> , internal jugular vein	<i>v.a.c.</i> , anterior cerebral vein
<i>mes.</i> , mesoderm	<i>x.</i> , attenuated strips of endothelium
<i>m.l.</i> , muscular layer	

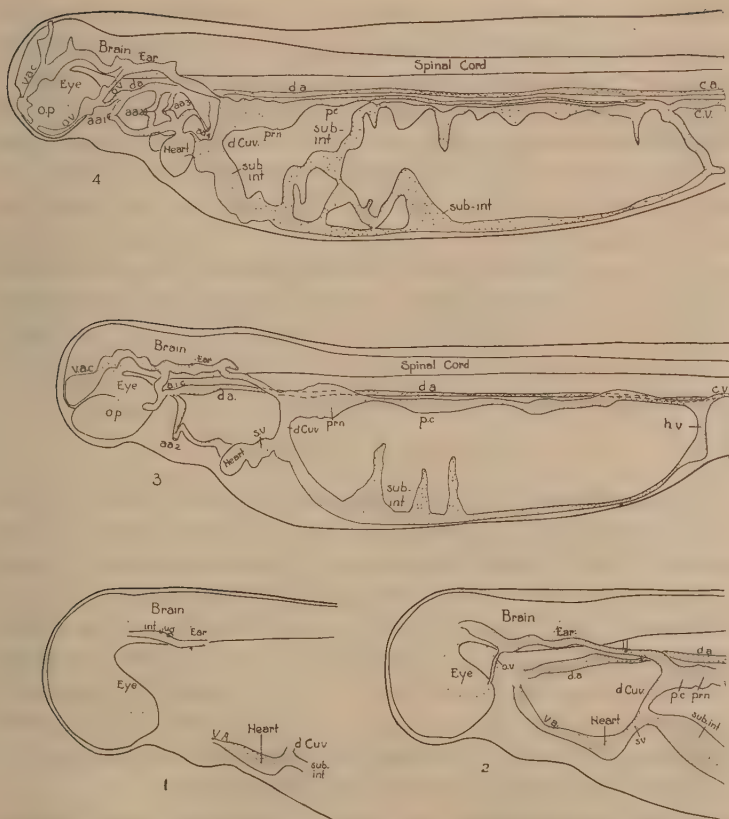


Fig. 1 A projection of the head of *Amblystoma punctatum* (specimen no. 469) of the non-motile stage upon the sagittal plane, to show the vascular system in its relation to the central nervous system. Made by the conventional methods from serial transverse sections $10\ \mu$ in thickness at a magnification of 100 diameters; reduced to $33\frac{1}{3}$.

Fig. 2 A projection of the head of *Amblystoma punctatum* of the early flexure stage (specimen no. 473) upon the sagittal plane, to show the degree of development of the vascular system. Methods same as for figure 1.

Fig. 3 A projection of the head and trunk of *Amblystoma punctatum* (specimen no. 449) of the coil stage upon the sagittal plane, to show the degree of differentiation of the vascular system. Methods same as for figure 1.

Fig. 4 A projection of the head and trunk of *Amblystoma punctatum* (specimen no. 444) of the early swimming stage upon the sagittal plane, to show the degree of differentiation of the vascular system. Methods same as for figure 1.

The dorsal aorta (*d.a.*) is definitely organized in the cephalic part of the trunk and extends caudad as far as the fourteenth myotome. The dorsal aortae of the branchial region can be traced cephalad below the level of the auditory vesicle. The posterior cardinal veins (*p.c.*) are seen in connection with the pronephros (*prn.*) as fairly definite blood spaces. The ductus Cuvier (*d.Cuv.*) and the subintestinal vein (*sub.int.*) join to become the sinus venosus (*s.v.*). The main cardinal vessels pass cephalad to the caudal border of the mesencephalon. These internal jugular veins (*i.j.*) send their ophthalmic branches (*o.v.*) to the ventral surface of the brain caudally of the optic vesicle, but there is no perceptible connection between them and the arterial system.

3. The coil stage (fig. 3)

In the coil stage the heart has become more coiled than in the last stage, but it is still a simple, undivided tube. A connection is now established between the afferent and efferent vessels of the first gill, forming the second aortic arch, the first aortic arch forming later in the balancer. The dorsal aorta extends caudad as far as the twenty-first myotome, where it joins the caudal vein (*c.v.*). Beyond this point of union a blood space extends to the level of the twenty-ninth myotome. The dorsal aortae unite to form the dorsal aorta at about the level of the caudal end of the heart.

Around the tubules of the pronephros (*prn.*) are definitely organized blood spaces which communicate with the sinus venosus (*s.v.*) through the cardinal system and ductus Cuvier (*d.Cuv.*). The posterior cardinal veins (*p.c.*) continue caudad laterally of the wolffian ducts. The hypogastric vein (*h.v.*) extends ventrad from the postcardinal at about the level of the eighteenth myotome and unites with the large subintestinal spaces, which represent the subintestinal vein (*sub.int.*). These spaces spread widely over the ventrolateral surface of the yolk and collect anteriorly into the sinus venosus.

The internal carotid artery (*a.i.c.*) has grown cephalad beneath the optic vesicle until it makes connection with the ophthalmic vein.

The cephalic part of the anterior cardinal vein now spreads out over the surface of the brain laterally and becomes a broad vessel around the anterior end of the diencephalon, forming an anterior cerebral vein (*v.a.c.*). There is also a branch of the internal jugular vein which passes caudad from the region of the auditory vesicle between the nervous system and the first myotome.

4. *The early swimming stage (fig. 4)*

The coiled condition of the heart is more pronounced than in the last stage, but otherwise this organ has undergone no fundamental changes in gross structure. The afferent and efferent vessels of the balancer have now anastomosed to form the first aortic arch, and the third and fourth aortic arches are completed in the second and third gills, respectively (*aa.* 1, 3, 4). The anterior cerebral veins (*v.a.c.*) now extend to the rostral end of the brain and encircle the paraphysis. Each has a dorsal branch, also, which anastomoses above the isthmus with the corresponding vessel of the opposite side. The ophthalmic vein is very closely associated with the internal carotid artery and passes forward till it nearly reaches the anterior cerebral vein.

During this stage suggestions of segmental veins appear on the medial side of the myotomes next to the notochord and situated in the angles between the myotomes. These segmental veins appear first in the vicinity of the first myotome and differentiate caudally in the later stages. The connection of the caudal vein (*c.v.*) and artery (*c.a.*) is not made till the level of the thirtieth myotome is reached. The blood space caudal to this union extends to the thirty-first myotome. The irregular blood spaces which constitute the subintestinal vein on the ventrolateral surface of the yolk are still large and have become still more complex. The relations of the posterior cardinal veins with the pronephros remain as in the coil stage.

B. HISTOLOGICAL COMPARISON OF STAGES

1. *The heart*

The heart in the non-motile stage of the embryo is a simple tube of mesoderm in two layers, of which the inner is destined to become the endothelial tube and the outer, the muscular wall of the organ (fig. 5; *end.*, *ml*). The pericardial cavity (*pr.c.*) is clearly defined (fig. 5). The yolk content of the cells of the heart as compared with those of the skin is shown in figure 5. The yolk globules in the cells of the heart are much larger than those in the skin, and the mass of yolk as compared with the size of the cell is much greater in the heart than in the skin.

There is no noteworthy change in the histological structure of the heart of the early flexure stage as compared with the non-motile stage (fig. 6) excepting that in the endothelial tube of the early flexure stage there are small patches of exceedingly thin membrane (fig. 6, *x*) connecting the thicker cell masses. In the coil stage the yolk-free areas of endothelium (fig. 7, *x*) are much more extensive and the yolk is very much reduced in amount in both the endothelial and muscle layer. In the transition to the early swimming stage the change has gone much farther in this respect (fig. 8). The cells of the endothelium have relatively little cytoplasm and appear much as do the endothelial cells of veins and arteries (fig. 13).

The muscular character of the heart is only beginning to show histologically in larvae several days past the early swimming stage (fig. 9, *mf*). At this time only small yolk globules occur rarely in the endothelial layer (fig. 9, *end.*) and there are very few yolk globules in the muscular layer. This is in accord with the observation of Hertwig ('92) that there are no muscle fibrillae in the myocardium when it begins rhythmic contraction.

2. *The blood vessels*

In the non-motile stage the dorsal aorta is completely formed in some regions, but not throughout its entire extent



Figs. 5 to 9 Sections through the heart of *Amblystoma*. Figure 5, non-motile stage; figure 6, early flexure stage; figure 7, coil stage; figure 8, early swimming stage; figure 9, stage of several days after swimming began.

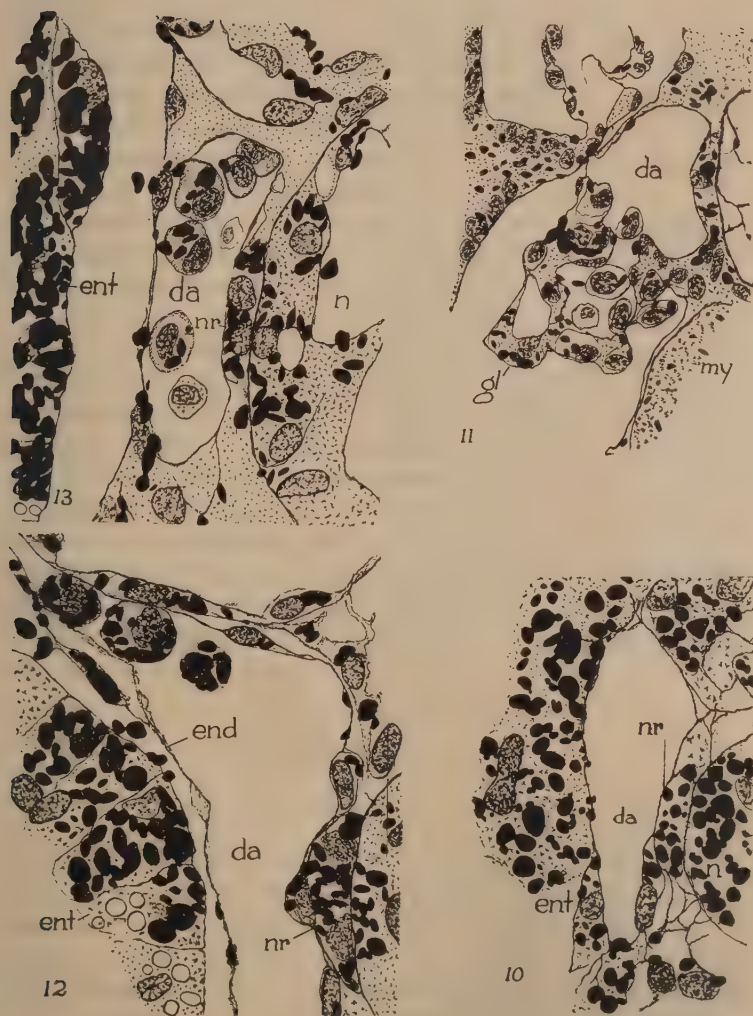
(fig. 10, *da*). In the early flexure stage it is a continuous vessel of endothelial cells (fig. 11, *da*). There is only an occasional yolk globule in the endothelium in the more rostral part of the vessel, but the yolk is much more abundant in the more caudal part. Opposite the pronephros is a sacculated glomerular (fig. 11, *gl.*) outgrowth which has the structure of an incipient glomerulus. In the coil stage other blood vessels, both veins and arteries, are essentially like the aorta in the thin endothelial structure of the wall (fig. 12, *da*). The smaller vessels, however, have more yolk in proportion to the cell mass than do the larger vessels. In the early swimming stage the dorsal aorta has acquired a more definite and regular contour in cross-section (fig. 13, *da*), but it is still comprised exclusively of endothelial cells. This is true also of all the other blood vessels.

In all of these stages of development there is ventrally of the notochord in the trunk region a strand of cells which Marshall ('93) calls the subnotochordal rod (figs. 10, 12, 13, *nr*). This enters into the structure of the wall of the aorta dorsally.

3. *The corpuscles*

In the non-motile stage the blood corpuscles are masses of irregular contour and so filled with yolk globules about the nucleus that there is very little visible protoplasm in them. They occur in the lateral and ventral parts of the animal and in spaces either in the mesoderm or between the mesoderm and the entoderm in that region. In the non-motile embryo these seem to be closed spaces without intercommunication. They form large indentations into the entoderm. Dense cell masses bordering these spaces may be regarded as blood islands. Corpuscles have been observed that appear to be breaking off the entoderm and wandering into the spaces.

In the early flexure stage these ventral and lateral blood spaces have become continuous. The corpuscles have not changed perceptibly in structure, but are now seen also in the sinus venosus and the heart (fig. 6). In the coil stage



Figs. 10 to 13 Sections through the dorsal aorta of *Amblystoma*. Figure 10, non-motile stage; figure 11, early flexure stage; figure 12, coil stage; figure 13, early swimming stage.

there has been a marked reduction of yolk in the corpuscles, and they have acquired a more even, rounded contour (figs. 7 to 12). The protoplasm is now clearly defined about the yolk globules which are grouped particularly in the vicinity of the nucleus. Many corpuscles that are in circulation are undergoing mitosis. In the early swimming stage the yolk in the corpuscles has become much more reduced in amount and many corpuscles show a regular oval contour approaching their definitive form (figs. 8, 3). Many yolk globules lie in pits on the surface of the nucleus as if attacked by amoeboid action on the part of the nucleus.

Upon measurement, the greatest length of the corpuscles in the early swimming stage averages about the same as does the diameter of the corpuscles in the early flexure stage. In assuming the ovoid form there has been, therefore, a reduction in volume. This change accompanies the reduction in the amount of yolk. The average diameter of the corpuscles in the coil stage is 0.0214 mm. The corpuscles of the adult *Amblystoma* average about 0.023 mm. in length, but are much more flattened and oval in form. The corpuscles of the stages under consideration are probably all erythroblasts or erythrocytes. The red color in the living corpuscles is visible the second day after swimming begins.

C. PHYSIOLOGY

1. *The heart beat and circulation*

The rhythmical contraction of the heart begins during the early flexure stage of development. No circulation of corpuscles, however, can be seen in the blood vessels until the coil stage. If there is a rhythmic contraction of the heart previous to the early flexure stage it cannot be detected with the aid of either the compound microscope or the binocular dissecting microscope.

The rate of the earliest heart beat noted was twenty per minute, but the average rate for all embryos of this stage (from 6.25 to 6.5 mm.) was thirty-five per minute. In the

embryo of the coil stage the rate varied from forty-two to sixty-three per minute (average length 6.5 mm.). In the early swimming stage the rate varied from forty-nine to seventy-two (length 6.25 to 8 mm.). All of these observations were made upon *Amblystoma microstomum* Cope.

Prolonged tactile stimulation has no perceptible effect upon the rate of heart beat. Neither do moderate changes in the general environment. However, when death is brought about by exposing the animal in too shallow water so that the gills slowly become dry in the atmosphere or by any other slow method the rate of heart beat is at first accelerated and then retarded. Also, for a variable period immediately before complete cessation of the blood flow, there is a backward flow of the blood at diastole. In the case of one specimen that had been sealed in a tube of 2 cc. of water the backward flow of the blood in the gills at diastole began after about forty-eight hours of confinement. It continued for about three hours, when the blood flow ceased entirely. The embryo was then removed from the sealed cell and placed in fresh water. The heart then began to beat again, but with backward flow of blood at diastole. The normal flow was later established.

2. The nervous system

There are no blood vessels in the central nervous system until several days after the early swimming stage. Veins develop, however, very near the surface of the brain in the very early stages. The earliest to acquire definite form are the anterior cardinal veins, which lie very close to the brain along the lateral surface of the medulla oblongata from the level of the auditory vesicle to about the isthmus (fig. 1). They grow rapidly cephalad and caudad till in the early swimming stage they have reached the end of the brain rostrally and the full extent of the medulla oblongata caudally.

3. Muscles

There are no blood vessels in the myotomes up to and including the early swimming stage, but in the latter stage,

segmental veins have begun to differentiate in the angles between the myotomes medially. The circulation of corpuscles in these veins, however, is not visible till the second day after swimming begins, although on the first day after swimming begins an occasional corpuscle is found here in sectioned specimens. Circulation in the segmental system begins first in the rostral part of the trunk. In an embryo that had been swimming for four or five days circulating corpuscles are visible around the caudal myotomes, and very soon after this capillaries appear in the dorsal and ventral fins of the tail. The growth of the capillary net in the tail, which appears in the anuran embryo during the first week of development (Clark, '09), does not appear in embryos of *Amblystoma microstomum* until the end of the second week.

4. *The gills and balancers*

There is no perceptible circulation of blood through the gills in the coil stage. Some embryos of the 'S' reaction stage, but not all, have blood circulating through the gills, while circulation is established in the first and second gill in all early swimming embryos. In some the blood is circulating through the third gill also, and, a few hours later, it begins to flow through the balancers.

CONCLUSIONS

1. Since, *a*) all cells of the body are supplied with intracellular food material and, *b*) the nervous and muscular systems are not penetrated by the vascular system, and, *c*) the alimentary system is in a very low stage of differentiation and functionless, the vascular system of *Amblystoma* up to and including the early swimming stage can have no importance as a mechanism of nutrition.

2. In respect to its aerating function, although the heart begins to beat in the early flexure stage, the vascular system probably begins to play a part in the elimination of carbon dioxide from the deeper tissues, particularly in the vicinity of the descending aorta and cardinal veins, anterior and

posterior, in the coil stage, when the vascular circuit is completed in the first gill, between the descending aorta and the venous system and between the internal carotid artery and the ophthalmic vein; but it is probably not an important specialized carrier of oxygen till the corpuscles acquire haemoglobin sufficient to give them perceptible color, some two days after the early swimming stage.

3. Although there is a glomerulus upon the descending aorta in the early flexure stage, this organ could not have important excretory function till the coil stage, when the vascular circuit becomes complete; but since the heart beats during the early flexure stage, body fluids may at that time be collected through the anterior cardinal system and carried at least into the sinusoids around the pronephric tubules, which, if functional at that time, could possibly have important action upon the body fluids.

4. Up to and including the early swimming stage, the vascular system probably functions as a mechanism of excretion only, and it becomes important in this respect when, in the coil stage, the muscular system acquires the power of tetanic contraction.

5. Correlated with the acquisition of locomotion by the embryo are, 1) the completion of the vascular circuit through all the gills and the balancer, 2) increased rate of heart beat, and, 3) marked change in the shape of the corpuscles toward the characteristic definitive form.

DISCUSSION

1. The behavior pattern of the embryo

The development of the behavior pattern of *Amblystoma* up till swimming begins has been described and discussed in several contributions by the senior author (Coghill, loc.cit.), but possibly sufficient emphasis has not been placed upon the strictly embryonic nature of the behavior under consideration. When embryos of *Amblystoma* break through the egg membrane and begin their strictly larval existence they

swim perfectly and with great strength immediately upon liberation into the water. The swimming mechanism, therefore, has grown into a perfectly coordinated dynamic system without the locomotive value of its function per se having anything to do with it as a regulating factor in development. Nevertheless, there are important structural developments apart from the swimming mechanism itself that are distinctly correlated with the beginning of the ability to swim and that appear to be distinctly adaptive with reference to locomotion. The senior author (Coghill, '24 a, '24 b) has already discussed some of these, i.e., the growth of the nerves from distance receptors into the brain, the relative acceleration of the differentiation of neuroblasts out of indifferent cells in the cerebrum, that part of the brain which especially enables the animal to use the varied environment that locomotion introduces, etc. So, also, in these studies on the vascular system, it is found that features arise with the beginning of swimming that appear to have adaptive value with reference to locomotion, although the animal is normally confined within the egg membrane at that time and cannot for that reason actually propel itself into new environment, by means of its functional mechanism of locomotion. The most noteworthy of these features is the completion of the vascular circuit through the gills and balancers, the specialized organs for aeration of the blood, the function of which would be facilitated and amplified by locomotion, which itself calls for increased capacity for aerating the tissues. There should be mentioned in this connection, also, the marked change in the form and consistency of the blood corpuscles toward their definitive condition during the transition period from the coil to the early swimming stage and the marked acceleration in the rate of the heart beat. So that between these several features and the perfection of the mechanism of locomotion there is a very definite correlation, although locomotion is normally impossible because of the confinement of the embryo within the egg membrane. While analogous correlations that need not be discussed in detail are commonly recognized in a gen-

eral way in the hatching, birth, or metamorphosis of many animals, these correlations that have been noted in *Amblystoma* have been determined in an exact way by the detailed study of structure and function in development, and give certain definite data upon the basis of which to evaluate the performance of a function per se as a factor in the development of behavior.

A similar correlation may be observed in the coil stage. This stage, as already noted, is characterized by prolonged tetanic contraction of the muscles on one side of the body only in any particular movement. This movement may be reversed and a similar movement may be executed on the side opposite the first. Indeed, a series of these tetanic contractions in alternating directions may occur till the animal appears to be exhausted. The neuromuscular system, therefore, at this time has acquired the capacity for the expenditure of relatively great muscular energy. It is noteworthy, therefore, that it is at this time that the vascular circuit has first become established through the trunk and through one of the aortic arches.

2. Tissue differentiation and the development of veins

The earliest blood vessel to take on definite form, excepting the most basal parts of subintestinal vein and ductus Cuvier, is that part of the anterior cardinal vein which lies alongside of the medulla from the ear vesicle to near the isthmus. It is particularly noteworthy that this vessel develops very close to the surface of the brain, excepting as it is crowded farther ventrad by the auditory vesicles. This relation is seen in the non-motile stage (fig. 1, *int.jug.*). In the transition period between the non-motile and early flexure stage the vessel pushes farther dorsad between the auditory vesicle and the brain and establishes connection caudad with the posterior cardinal vein. Caudally of the auditory vesicle for a considerable distance it clings closely to the brain before it leaves this position for a more direct course to the posterior cardinal vein and ductus Cuvier. In the coil and early swim-

ming stages this vein develops progressively more extensive close relation to the brain caudally. Now, according to the observations of the senior author (Coghill, '24 b) upon rates of differentiation in the central nervous system, this anterior cardinal vein follows a course directly over the regions of most rapid differentiation of neuroblasts from indifferent cells. In the more rostral development of this vein in the coil and early swimming stages, also, it follows definitely the regions of rapid differentiation in the nervous system, the lateral aspect of the medulla oblongata and the mesencephalon, and around the hypothalamus in the coil stage and to the olfactory region in the early swimming stage.

It appears that vertebrates generally are essentially like *Amblystoma* in regard to the site of earliest differentiation in the central nervous system. According to Cowdry ('14), the earliest neurofibrils in the chick embryo develop in three chief localities: "(1) in the hind brain opposite the otic invaginations; (2) in the nuclei and the root fibers of the cranial nerves; and (3) in a center on either side of the extreme anterior end of the midbrain." Also Tello ('23), in his extensive study of the development of conduction paths in the central nervous system of the chick by the method of silver impregnation of Cajal, finds that the earliest outgrowth of fibers takes place in the basal plate near the sulcus limitans in the diencephalon and along the corresponding course in the rhombencephalon to the rostral part of the spinal cord. There is a correspondingly close agreement between vertebrate types in regard to the site of development of the earliest veins about the central nervous system.

With reference to the posterior cardinal veins, it should be observed that they develop in close relation to the ventral part of the myotome, which in *Amblystoma*, according to studies by Faris ('24), is the most rapidly differentiating part of the muscular system. Furthermore, according to the observations of Faris ('24) upon pigment in *Amblystoma*, differentiation in the muscular and nervous system produces characteristic substances, some of which are not eliminated

from the tissues until the vascular system grows into them. The idea very naturally suggests itself, therefore, that products of differentiation metabolism in the central nervous system and in the myotomes activate the differentiation of venous channels.

This suggestion, however, does not seem applicable to the subintestinal vein, for it develops through the region of least differentiation in the embryo. But this vein arises in essentially hemapoietic tissue and may, therefore, be subject to peculiar factors of regulation.

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A NOTE ON THE CONNECTIONS, IN THE MAMMALIAN MYENTERIC PLEXUS, BETWEEN THE ENTERIC NEURONES AND EXTRINSIC NERVE FIBERS

F. W. CARPENTER

Biological Laboratory of Trinity College, Hartford, Connecticut

The mammalian gastro-intestinal canal throughout the greater part of its course is supplied with efferent nerve fibers from two sources—the splanchnic nerves and the vagus nerves. The splanchnic fibers belong, in the terminology proposed by Langley, to the sympathetic or thoracolumbar division of the autonomic system. They are postganglionic and chiefly inhibitory in function. The vagus fibers belong to the cranial division of the autonomic system. They are pre-ganglionic and chiefly excitatory in function. Both sets of fibers enter the ganglionated nervous plexus between the longitudinal and circular muscles of the digestive tube, the myenteric plexus, or plexus of Auerbach. So far the facts are well known, but as to what becomes of these extrinsic fibers after reaching the myenteric plexus there is still uncertainty. Where and how do the fibers end? What are their anatomical relations with the intrinsic nervous elements of the plexus? Are these relations the same for both sets of extrinsic fibers or are they different for each set? To such questions the neurohistologist is still seeking the final answers. He has not yet been able to read under the microscope the riddle of the organization of the myenteric plexus. But from its microscopical study various facts have emerged which, together with the results of physiological experiments on the activities of the digestive tube, give us a basis for discussing some of the probable relationships of its elements. It is these relation-

ships that must be worked out if we are to understand the enteric nervous system as a functional mechanism.

As far as the problem of the termination of extrinsic efferent fibers in the enteric wall is concerned, it may be said, in the first place, that it seems probable that such fibers end within the limits of the enteric plexuses, and chiefly in the myenteric plexus. The assumption is that the extrinsic axones exercise their regulatory control over the activities of the digestive tube through making connection with the local nervous mechanism, and not through the direct innervation of the enteric effectors (muscles and glands) themselves. This view is supported by the histological observations of Cajal ('93) and Dogiel ('95, '99), whose descriptions of the enteric plexuses still stand as the most illuminating we possess. Both authors agree that the nerve fibers entering the myenteric plexus from outside terminate within it. The fibers distributed to the musculature of the digestive tube have been identified by all observers as the processes (axones) of the local enteric neurones whenever it has been possible to make out their proximal connections. Only on theoretical grounds has this view of the destination of all extrinsic fibers been questioned. Since the splanchnic fibers are postganglionic sympathetic axones, and are affected little or not at all by nicotine in the myenteric plexus, Langley ('22) considers it probable that they do not connect with enteric nerve cells. They pass on, presumably, to terminate, as do other postganglionic fibers of the autonomic system, in muscular or glandular tissue. The preganglionic vagus fibers, on the other hand, are believed by Langley to end on nerve cells within the enteric plexuses.

Secondly, it appears that extrinsic fibers may terminate in the myenteric plexus in two ways. They may end in pericellular end nets or arborizations (Dogiel, '95, '99; Huber, '97; De Witt, '00; Kuntz, '13) not differing materially from the characteristic intracapsular, pericellular baskets in which medullated preganglionic fibers terminate in vertebral and prevertebral sympathetic ganglia. Or they may end in a

simpler and less restricted manner by merely forking into a number of branches which end abruptly, often with slight expansions at their tips, on the dendrites or the cell bodies of the enteric neurones (Kuntz, '13; Glaser, quoted by Grev-ing, '20). Endings of this kind were apparently seen by Cajal ('93) and by Dogiel ('99), who described them as the terminations of exceedingly fine varicose fibers in the inter-cellular fiber complex or on the local ganglion cells. That such endings were observed by Sabussow ('13) in the inter-muscular plexus of the mammalian oesophagus may be inferred from his description, while their occurrence in ordinary sympathetic ganglia has been noted by von Lenhossék ('94) and by Huber ('97).

Endings of the first type, as Huber ('97) pointed out, are not abundant. Their number is relatively small as compared with the number of ganglion cells and of the ultimate branches of extrinsic fibers that must be present. This scarcity of pericellular endings in the myenteric plexus of the small intestine in various mammals (cat, dog, guinea-pig) the writer can confirm from his own studies. After making due allowance for the sporadic staining of methylene blue, by the use of which these pericellular structures have been demonstrated, the impression remains that very many of the enteric neurones are not connected by this kind of synapse with the extrinsic nerve fibers. Furthermore, some of the pericellular endings observed have been shown to be the terminations of processes of the local neurones (Dogiel, '95; Kuntz, '22).

On the other hand, the simpler endings of the second type are probably more numerous than has been recognized, especially those which occur on dendrites in the intercellular spaces. Here extrinsic fibers, which are non-medullated and varicose in this part of their course, and the quite similar processes of the multipolar local neurones are intermingled in a fiber complex, the intricacy of which, it is true, makes the identification of its different components no easy matter. In such an interlacement it is especially difficult to be certain of abrupt, undifferentiated fiber terminals, to be sure that

one has under observation the actual ending, the final varicosity, of a delicate nerve fiber in contact with a dendrite or the cell body of another neurone. Yet in spite of the possibilities of error in the interpretation of the picture, the number of apparently authentic examples of such terminations to be seen in methylene-blue preparations seems, in the writer's opinion, to warrant the view that many extrinsic fibers do end thus in the intercellular areas without forming cell-embracing nets or arborizations.

If two types of endings of extrinsic fibers are, then, present in the mammalian myenteric plexus, the third point to be considered is the relation of these endings to the two sets of fibers entering the plexus from without, assuming that both sets terminate therein. No direct microscopical evidence is here available. By mere inspection it is impossible to distinguish between vagus and splanchnic fibers within the limits of the plexus. But in this connection the observations of physiologists who have experimented on the movements of the intestine are of interest and perhaps significant, although we are here entering admittedly on questionable grounds for the drawing of inferences as to structural relations.

Bayliss and Starling ('99), studying the action of the vagus on the small intestine of the dog, found that its effect (at first inhibitory, but later predominantly motor) could be permanently suppressed by a small amount of nicotine applied to the enteric wall. On the contrary, Langley and Anderson ('95), experimenting with the splanchnic nerves, were unable to abolish with nicotine the action of these nerves (partly motor, but mainly inhibitory) upon the musculature of the lower intestine in the cat. The effects of stimulation were sometimes diminished, but never completely annulled.

Is it not possible that the explanation of this difference in reaction to nicotine lies in the difference in the kind of synapses which the fibers from the two sources make with the enteric neurones? Nicotine, as is well known, when applied to ordinary sympathetic ganglia, prevents the passage of nervous impulses through them, presumably by paralyzing

the delicate fibrillar end baskets of the preganglionic fibers, as Huber first suggested. If the fibers of the vagus nerves end in pericellular nets, it might be expected that nicotine would affect these nets just as it does those of sympathetic ganglia. The results of Bayliss and Starling could then be accounted for on histological grounds.

But if the fibers of the splanchnic nerves end by branching in the intercellular spaces, and establish widespread contact relations with the dendrites and bodies of enteric cells, one extrinsic axone perhaps being in functional connection with a number of local neurones, it is conceivable that we may have here a form of synapse more resistant to nicotine than the pericellular basket-cell body connection limited to two neurones only. The more diffuse intercellular synapse, possibly involving many dendrites and extended by contacts with cell bodies, may constitute a mechanism for nervous transmission so efficient that nicotine, while it may weaken, cannot abolish functional activity. Indeed, Langley and Anderson thought that the weakening of the effect of stimulation in their experiments might have been due to the action of the nicotine on the motor nerve endings in the smooth muscle. Even if synapses of the kind suggested are present, this might have been true, for Langley and Dickinson ('90) have shown that in mammals the axone-dendrite synapses in the spinal cord uniting the neurones concerned in ordinary spinal reflexes are at least as resistant to nicotine poisoning as the motor endings of the efferent fibers in skeletal muscle.

It is to be remembered that the splanchnic fibers are post-ganglionic, the second members of the typical sympathetic sequence of two efferent neurones. Their cell bodies lie in the prevertebral ganglia of the abdomen (Bunch, '98). Throughout the autonomic system it is the preganglionic fibers which end in pericellular synapses susceptible to the paralyzing action of nicotine. When Langley and Anderson failed to check completely with nicotine the discharge of nervous impulses through the synapses of splanchnic fibers in the myenteric plexus, they were doubtless correct in concluding that "these

connections are of a different nature from those which exist between pre-ganglionic fibres and sympathetic nerve cells."

Vagus fibers, on the other hand, are typical preganglionic components of the autonomic system (Langley). Their course is not interrupted until they reach the peripheral ganglia of the viscera which they supply. It might be expected that in conformity with other preganglionic fibers they would end in pericellular structures. In fact, Larsell ('21) and Larsell and Mason ('21) have found by direct observation, confirmed by degeneration experiments, that the fibers of the pulmonary branches of the vagus end in the rabbit's lung in typical pericellular networks enclosing ganglion cells. If the gastrointestinal fibers of this nerve end differently, they stand in contrast to the pulmonary fibers. While conclusive microscopical evidence on this point is still lacking, it is of interest to note that L. R. Müller ('10), on tracing branches of the vagus into the gastric musculature, found in their course groups of ganglion cells about some of which pericellular nerve nets were visible in microscopical preparations.

The small number of pericellular nets to be seen in the myenteric plexus of the small intestine as compared with the number of enteric ganglion cells has been mentioned. On the assumption that pericellular nets are the terminals of vagus fibers, their relative scarcity in the intestinal wall can be correlated with its meager innervation by that nerve. Langley ('22) comments upon the improbability of there being enough vagus fibers to run to all the nerve cells of the small intestine. He suggests, however, that those cells, comparatively few in number, on which the vagus fibers end, may give off processes with many collaterals, which in their turn form synapses with the remaining local neurones, none of which, according to his theory, are in functional continuity with splanchnic fibers.

SUMMARY

1. The extrinsic efferent fibers distributed to the gastrointestinal wall of mammals by the vagus and splanchnic nerves all end, according to the available histological evidence, in the enteric nervous plexuses.

2. In the mammalian myenteric plexus two modes of termination of extrinsic fibers have been described: *a*) pericellular nets or arborizations embracing the cell bodies of enteric neurones and, *b*) less specialized, abrupt endings of freely branching axones, which establish widespread contact relations with the dendrites and cell bodies of the multipolar enteric neurones. These endings are intercellular, not pericellular.

3. Certain observations, physiological and histological, offer indirect evidence suggesting that the preganglionic vagus fibers end in the pericellular structures, while the postganglionic splanchnic fibers terminate by branching in the intercellular fiber complex.

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THE APPEARANCE OF NISSL SUBSTANCE IN NERVE CELLS FOLLOWING VARIATIONS IN FIXATION

A. E. HOPKINS

Zoölogical Laboratory, Harvard University

INTRODUCTION

Recent researches on Nissl substance (Mott, '12; Marinisco, '12) have led to the view that it does not exist in living nerve cells as definitely formed bodies as had been more generally supposed. The use of vital stains and of other methods of studying living cells has failed to reveal any structures morphologically comparable to Nissl bodies as seen in fixed and stained material. It is now generally believed that these bodies are coagulated protein substances which normally exist in a more or less diffuse condition in the living cell (Bayliss, '20).

Nissl bodies are commonly observed as structures ranging in size and structure from diffuse, granular material to bodies of definite form and outline. Is it possible that this variation may depend, to some extent, upon the fixatives used? If this is true, it is desirable to know the effects of certain chemical agents used in the fixation of Nissl substance. Such knowledge might also give some indication of the condition of this material in the living cell.

I wish to express my thanks to Dr. S. R. Detwiler for suggesting this work and for his continual criticism and advice during its progress.

MATERIALS AND METHODS

All observations were made on the motor cells of the ventral gray columns in the spinal cord of the common adult white rat, *Mus norvegicus albinus*. No anaesthetic was used. The animals were killed by a blow on the head. Pieces of spinal cord were fixed in fluids which were arranged in groups as indicated in table 1. All tissues fixed in a group at a particular time were taken from the same animal. After fixation all pieces of tissue in a given group received identical treatment. Sections of tissues which were fixed in the particular fluids listed for each group were mounted together on slides and stained.

The fixatives in group 1 (table 1) are modifications of Zenker's fluid, which contains an aqueous solution of potassium bichromate, corrosive sublimate, and sodium sulphate, and to which 5 per cent of acetic acid is added at the time of using. The modifications were made by varying the amount of acetic acid as follows: no acetic, 2, 5, and 10 per cent. Tissues were fixed for twenty-four hours.

In group 2 (table 1) tissues were fixed for forty-eight hours in the following fluids: absolute alcohol, 95 per cent alcohol, 70 per cent alcohol, 95 per cent alcohol and 10 per cent formalin in equal parts, and 10 per cent formalin.

In group 3 (table 1) tissues were fixed for forty-eight hours in mixtures of alcohol and acetic acid as follows: 95 per cent alcohol, 95 per cent alcohol with 2, 5, 10, and 20 per cent acetic acid.

Group 4 (table 1) consisted of material fixed in formalin and acetic acid mixed as follows: 10 per cent formalin, 10 per cent formalin with 2 and 5 per cent acetic acid. Tissues were fixed for forty-eight hours.

Sections from all groups were stained with toluidin blue, thionin, and by Nissl's methylene blue-venetian soap method. In group 1 sections were also stained with iron haematoxylin to demonstrate mitochondria, and by the Macallum haematoxylin method to demonstrate the iron-containing proteins (Nicholson, '23).

The accompanying table shows the variations in size and structure as judged by their appearance in the great majority of cells.

In group 1, where Zenker's fluid without acetic acid was used for fixation, the resulting picture was a diffuse mass of very fine granular material existing throughout the perikaryon with the exception of the axon hillock. In certain cases

TABLE 1

Showing variations in size and definition of Nissl bodies following different fixatives. Relative sizes are represented by figures, 10 indicating the maximum

GROUP	FIXATIVE	SIZE	
1	a) Zenker (no acetic)		Finely granular; diffuse
	b) Zenker (2 per cent acetic)	6	Clearly defined
	c) Zenker (5 per cent acetic)	7-8	Clearly defined
	d) Zenker (10 per cent acetic)	8	Clearly defined
2	a) Alcohol, 100 per cent	9	Indefinite
	b) Alcohol, 95 per cent	9	Indefinite
	c) Alcohol, 70 per cent	9	Indefinite
	d) Alcohol, 95 per cent, 1 pt. Formalin, 10 per cent, 1 pt.	8-9	Very clearly defined
	e) Formalin, 10 per cent,	8-9	Indefinite
3	a) Alcohol, 95 per cent	9	Indefinite
	b) Alcohol, 95 per cent (2 per cent acetic)	10	Very clearly defined
	c) Alcohol, 95 per cent (5 per cent acetic)	10	Very clearly defined
	d) Alcohol, 95 per cent (10 per cent acetic)	10	Very clearly defined
	e) Alcohol, 95 per cent (20 per cent acetic)	10	Indefinite; granular
4	a) Formalin, 10 per cent	8-9	Indefinite
	b) Formalin, 10 per cent (2 per cent acetic)	8-9	Very clearly defined
	c) Formalin, 10 per cent (5 per cent acetic)	8-9	Very clearly defined

slight local concentrations of the material were observed, but very seldom did they occur as definite bodies. However, when acetic acid was added to the fluid, definitely formed bodies resulted and their size varied with the concentration of the acid. When 2 per cent acetic acid was used, the Nissl substance appeared as clearly defined bodies. Concentrations of 5 and 10 per cent of acid caused a marked increase in the size of the bodies, but did not alter their definition.

Alcohol, when used alone in concentrations of 70, 95, and 100 per cent, coagulates the substance into large bodies with rather irregular, indefinite boundaries. Ten per cent formalin gives a similar picture. On the other hand, when alcohol and formalin are mixed together in equal parts, the homogeneity of the structures is increased and they appear as sharply defined bodies which are almost as definite in outline as well-stained mitochondria or other cell organs.

When tissues are fixed in 10 per cent formalin containing acetic acid in concentrations of 2 and 5 per cent, the resulting picture resembles very closely that following fixation in 95 per cent alcohol and 10 per cent formalin in equal parts. The Nissl bodies are very definite.

The same effect, to a less degree, is also noted when alcohol containing from 2 to 10 per cent of acetic acid is used for fixing (table 1). However, when 20 per cent of acetic acid is added, the bodies lose their clear outlines and assume an indefinite, granular appearance.

The increase in size of the bodies with the increase in concentration of acetic acid is most striking in group 1. This is probably caused by the swelling effect of the acid.

Nicholson ('23), who used the Macallum method of unmasking and staining organically bound iron, showed that such unmasked iron in nerve cells gives the same picture as do Nissl bodies, except that some of the smaller bodies are not visible and that other stained bodies appear in the nuclei. In the present work this same method was employed and the results are in complete agreement with those of Nicholson.

To prevent possible confusion with mitochondria, sections fixed in Zenker's fluid containing 5 per cent or less of acetic acid were stained with iron haematoxylin. Mitochondria were demonstrated as small, spherical bodies evenly distributed or clumped in the periphery of the cytoplasm. Sections stained in iron haematoxylin and restained in any of the Nissl body stains showed Nissl bodies and mitochondria in the same cells.

DISCUSSION

It appears from the present observations that the size and structure of Nissl bodies are largely dependent upon the treatment of the cells in preparation. Such cells, on the one hand, may contain a diffuse mass of basophilic substance throughout the greater portion of the cytoplasm, with no bodies appearing as distinct units. On the other hand, the cytoplasm may contain definitely formed bodies.

Whereas neither alcohol nor formalin, when used alone for fixation, will fix Nissl bodies as very clearly defined structures, the two substances together give compact, homogeneous, clearly delineated bodies. Acetic acid, added to Zenker's fluid, alcohol, or formalin (table 1), causes the Nissl substance to appear as more definite bodies than would be the case if the acid were not present. It is well known that protoplasm, when fixed, may give entirely different pictures according to the fixing agents used. The best fixing agent can do no more than give a rough approximation of the state of protoplasm in the living condition. In the present case it is difficult to determine which of the several pictures presented by Nissl substance most nearly resembles its condition in the living cell.

In the case of such cytoplasmic structures as mitochondria, evidence from fixed material is readily checked by vital staining methods. Nissl bodies, however, have not been demonstrated in living nerve cells. The evidence of Mott ('12) and of Marinesco ('12), that Nissl bodies do not appear in living nerve cells under dark-ground illumination, seems to indicate that they do not exist as such, and that their characteristic appearance when coagulated is an artefact dependent on the agents used for fixation. The results of ultramicroscopic examination, however, are not necessarily conclusive, for this method depends upon differences in the physical properties of the protoplasmic structures. It is possible that Nissl substance may not differ markedly in physical qualities from the surrounding medium.

The results recorded in groups 1, 3, and 4 show that the use of acetic acid as a constituent of the fixing fluids causes Nissl substance to be coagulated into structures of definite form and size. The quality of acetic acid which causes it to be used in most fixing fluids is its ability to bring about quick penetration and consequently fix structures in as near their natural form and position as possible. If this is its effect in the present case, it would appear to indicate that Nissl substance does occur as more or less clearly delineated bodies. However, since this evidence is based entirely upon observations of coagulated protoplasm and since Nissl bodies have not been observed in living nerve cells, such a conclusion would seem to be unwarranted.

It would appear as though Nissl substance were rather to be looked upon as a material not of a completely diffuse nature, but existing in the cell in localized concentrations. The variability of the pictures as obtained in the present preparations seems to be correlated with the variability in the coagulation of the substance. These observations would indicate that Nissl bodies are not definite structures comparable to mitochondria, but that their characteristic form is due to greater concentrations of the material in certain places.

SUMMARY

1. The appearance of Nissl substance in fixed preparations of nerve cells is largely dependent upon the substances used for fixation.

2. Zenker's fluid, without acetic acid, shows it as a diffuse, granular substance in the cytoplasm. In some cells, after such fixation, slight concentrations of the material are observable.

3. The presence of acetic acid in the fixatives used causes Nissl substance to appear as well-defined bodies which, in group 1 (table 1), increase in size with the amount of acetic acid used, up to 10 per cent.

4. Formalin and alcohol used together (table 1) give more clearly defined bodies than either of these substances used alone.

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NOTES ON THE INDEX OF THE FOOT AMONG YOUNG WHITE MEN

R. BENNETT BEAN AND CALVIN T. BURTON

Laboratory of Anatomy, University of Virginia

ONE CHART

The material for this study consists of 248 soldier engineers of the Truck Camp at the University of Virginia and 193 students at the university, examined and measured by the senior author during the World War. The anatomic type of each person was found by an examination of the ear, nose, face, and general body form. Among the measurements made at that time were stature, sitting height, foot length and breadth. From these the sitting-height index and foot index are obtained.

The foot index is the greatest breadth times 100 divided by the greatest length. A high index means a relatively broad foot and a low index, a relatively narrow one. The sitting-height index is the sitting height times 100 divided by the stature. A high index means a relatively high sitting height and relatively short legs and a low index the reverse. The sitting-height index will be called the body index.

A table has been prepared to show the correlation of body index and foot index in soldiers and students according to type. A table prepared in this way gives a four-dimensional view of the material and has been found useful in other work at present under way. The table has been divided into rectangles by lines drawn arbitrarily, so that the differences might be visualized without great difficulty, and the large numbers in the corners represent the totals in each rectangle.

51.5	Soldiers Students ¹	12 12	43 27	67 45	26 21	23 34	12 1	1	123 20	1856.0 1360.6	44.0 39.4	58.0 42.0	74
51.	Soldiers Students ¹	1 41	11 24	53 44	45 11	14 23	4 11	2 1	15 14	1649.1 1745.0	50.9 55.0	46.8 53.2	62
50.5	Soldiers Students	11 1	3 13	42 11	31 11	12 2	1 11	1	7 9	1236.8 469.3	63.2 30.7	50.0 50.0	32
50.	Soldiers Students	1 1	2 3	23 1	13 21	12 11	2 1		12 2	474.9 918.1	25.1 81.9	51.8 48.2	27
49.5	Soldiers Students	11 11	31 22		2 2	1 1	1 1		4 3	544.4 633.3	55.6 66.7	38.8 61.2	18
49.	Soldiers Students	2		2 1	2				2	5			7
48.5	Soldiers Students	104 1	1 1	1 1					121 2				3
Per cent Total	Soldiers Students Total	50.0 22.0 33.3	50.0 78.0 66.7	31.5 52.0 39.9	68.5 48.0 60.1	32.6 50.0 41.4	67.4 54.3 58.6	53.4 44.0 50.6	46.6 56.0 49.4	62.5 62.9 55.5	37.5 37.1 44.5	75.8 90.0 91.6	7.2 10.0 8.4
		7	58	87	65	60	24	8	2				441
Right foot index		34	35	36	37	38	39	40	41	42	43		

¹ Omitted from this chart but included in totals: 2 hypermorph students with body index 48 and foot index 37; 1 mesomorph student with body index 57 and foot index 39.

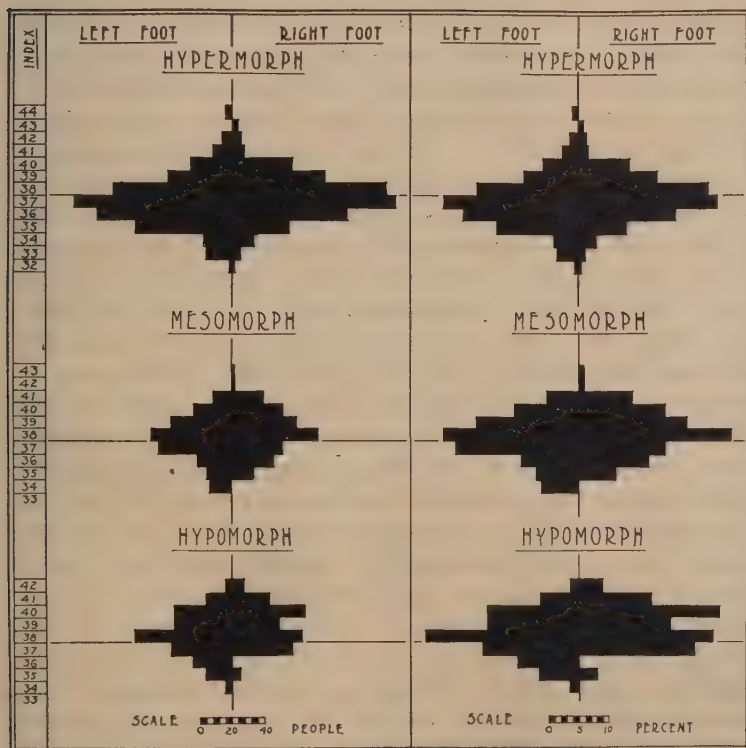
A distinct but not a great correlation between the two indices may be seen. This may be shown by comparing the numbers in the lower left and upper right corners of the table, $104 + 136 = 240$, with those in the lower right and upper left corners, $121 + 80 = 201$. It is seen that more persons with small body and foot index, or with large body and foot index, than with large body and small foot index, or small body and large foot index are to be found among the students and soldiers examined. From another standpoint, relatively long legs and narrow feet are found together more often than relatively short legs and narrow feet; and relatively short legs and broad feet are found together more often than relatively long legs and broad feet. It is evident that the body index increases with increase of foot index, but not in the same proportion or at the same rate.

Eighty-three soldiers and 52 students have relatively short legs and broad feet, whereas 47 soldiers and 57 students have relatively long legs and narrow feet. This may be explained by the greater number of mesomorphs than hypermorphs among the soldiers and the greater number of hypermorphs than mesomorphs among the students. Hypermorphs have lower foot and body indices than mesomorphs. The proportions here are as follows: Foot index 35: mesomorphs 33.3 per cent, hypermorphs 66.7 per cent; foot index 41: mesomorphs 91.6 per cent, hypermorphs 8.4 per cent. Body index 49.5: mesomorphs 38.8 per cent, hypermorphs 61.2 per cent; body index 53.5: mesomorph 62 per cent, hypermorph 38 per cent. The percentages change gradually from one extreme to the other between indices more extreme than these.

The soldiers have more mesomorphs than hypermorphs at each foot index and the students have more hypermorphs than mesomorphs, except at indices 36 and 37, where the reverse is true. Likewise with the body index, except at indices 50.5, 51.5, 52.5, and 53.5. We may conclude from this that the student is more slender and the soldier more stocky. Also that the foot index is a better differentiator of anatomic types among young white men than the body index. The latter

point may be emphasized by the greater differences in the proportions of the foot index than in the body index, at 35 and 41 foot index and 49.5 and 53.5 body index, as pointed out above.

A further division of the types was made by separating out the extreme mesomorph and calling it the hypomorph.



With this separation a chart was constructed to show the differences by actual numbers and percentages between the right- and left-foot index in the three types.

By the line below index 38 it may be seen that a larger percentage is below than above in the hypermorphs, while a larger proportion is above than below in the mesomorphs and hypo-

morphs. A line also separates the right- from the left-foot index, and it may be seen that a larger number of high indices are found for the right than for the left foot, and a larger number of low indices for the left than for the right foot.

The types were again divided by separating the extreme hypermorphs from the other hypermorphs, and by subdividing the extreme mesomorph. In this way five types were segregated. This was done at the time of the World War when the soldiers and students were measured and examined. A more minute subdivision might have been accomplished, but it was thought unnecessary. Curves were constructed by plating foot length as ordinate against foot breadth as abscissa for each foot separately and keeping the right and left feet apart. The number of each kind of foot to the right of the curve was compared with the number of the same kind to the left with the following results: Extreme hypermorph, right 78.6, left 100; hypermorph, right 76.9, left 100; mixed type, right 108.0, left 100; mesomorph, right 167.7, left 100; extreme mesomorph, right 425.0, left 100. This gives the relative breadth of the foot in terms of the length by individual count, because the hypermorph has three-fourths as many broad as narrow feet, whereas the mesomorph has four and a quarter times as many broad as narrow feet. It was also noticed that in the hypermorph the right foot is more often narrow than the left, whereas in the mesomorph the left foot is more often broad than the right. This but emphasizes that each type is extreme in its tendency to broad or narrow feet. The proportion in the extreme hypermorph is 72.1 to 100 for the right foot and 78.7 to 100 for the left foot, whereas in the mesomorph it is 250.0 to 100 for the right foot and 600.0 to 100 for the left. The difference in the hypermorph is less than 10 per cent, but in the mesomorph it is more than 100 per cent. This is because the hypermorphs were not separated as were the mesomorphs. There are only 21 extreme mesomorphs, but 104 extreme hypermorphs.

CONCLUSIONS

The foot index is slightly correlated with the body index and more closely correlated with the anatomic type.

The foot index of the hypomorph is high, that of the hypermorph is low, and that of the mesomorph is in between, but nearer the former than the latter. *

The foot index of the students is lower than that of the soldiers, and the students are more hypermorph, whereas the soldiers are more mesomorph.

The mean index of the right foot is higher than that of the left; it is therefore relatively broader, and this is true in all of the anatomic types.

The index of the right foot plus the index of the left foot results in a slightly lower index than that of the head, with a range between 65 and 85.

THE CRANIAL SUBDURAL SPACE

(A METHOD OF STUDY)

WILDER G. PENFIELD

*Department of Surgery, College of Physicians and Surgeons, Columbia University,
New York*

Pathological accumulation of fluid in large quantity between the dura mater and arachnoidea was described in an earlier communication.¹ This fluid was found to have a higher specific gravity and higher concentration of protein than the cerebrospinal fluid, the two fluids being obtained simultaneously. The concentration of easily diffusible substances such as glucose was identical in the two specimens, while their cellular content was always different. During this time drainage of the cerebrospinal fluid continued normally in the sub-arachnoid spaces beneath the subdural collection.

Interest in the condition described above led to study of the relation between the dura and arachnoidea in animals. An attempt was made to induce a fluid exudate beneath the dura by various inflammatory and infectious agents placed on the outer surface of the dura. Details of these experiments will be omitted, as little if any increase was caused in the subdural fluid. The following description applies to the normal animals used as controls.

Is the space between dura and arachnoid a truly potential space or does it normally contain fluid? To answer this question an attempt was made to aspirate fluid with fine needles and likewise to inject dye beneath the dura. It was impossible to exclude the presence of cerebrospinal fluid as the result of injury to the pia-arachnoid by this method. Consequently, the technique described below was used.

¹Penfield, W. G. Subdural effusion and internal hydrocephalus. *Am. Journ. Diseases Children*, vol. 26, pp. 383-390, 1923.

A 10 per cent solution of formalin containing a physiological concentration of sodium chloride was injected into the internal carotid of an anesthetized dog. A pressure sufficient to overcome the blood pressure in this artery was used, i.e., about 200 cm. The animal was taken at once to the refrigerator and frozen at a temperature a little below 0°C. At the end of five to fifteen days the head was sawed into slices by parallel cuts. By gentle pressure, the segment of brain included in a slab could then be pushed out. The pia-arachnoid, frozen in its layer of cerebrospinal fluid, was carried out with the brain segment. A layer of clear yellow ice was then found on the under surface of the dura or loosely applied to the outer surface of the arachnoid.² The cut surface of the brain presents minute cavities.

This amber-colored sheet of ice is thus found over the convexity of both cerebral hemispheres. It is entirely free. Its outer surface, corresponding to the dura, is smooth and glistening. Its under surface, which is in relation with the arachnoidea, is not so smooth, but minutely pitted. This ice sheet was often found to be 0.5 to 1 mm. in thickness over the convexity of the cerebrum, but usually thin beneath the falx. Considerable variation in the amount of subdural fluid exists, even in normal dogs. If the ice crystals be picked up and placed in a bottle, the resultant fluid varies from a drop or two to 1 cc. in dogs of 5 kilograms.

It is difficult to demonstrate the subdural ice over the cerebellar arachnoid in some animals, but in others beautiful clear crystals can be lifted with the forceps from the under surface of the tentorium. No similar layer of fluid was demonstrated beneath the spinal dura over the cervical cord.

The appearance of the frozen layer of fluid in the pia-arachnoid is different from the above. It is colorless. Incorporated in it are the trabeculae of the pia-arachnoid, and

² When the animal is frozen without preliminary formalin injection, as was done by Austmann and Moorhouse, it is not possible to push out the brain slabs in this way nor to obtain a line of cleavage on the outer surface of the arachnoidea. Austmann, K., and Moorhouse, V. On the function of the cerebral ventricles. *Am. Journ. Physiol.*, vol. 66, pp. 267-274, 1923.

even from the cisterna cerebello-medullaris the ice cake can only be removed by tearing it free from its membranes. The difference in color indicates that the subdural fluid does not take part in the cerebrospinal fluid circulation.

Finally, the cerebral and cerebellar subdural space is more than a potential cavity in normal dogs. It contains a variable amount of clear yellow fluid which is distinct from the cerebrospinal fluid.

